the cleavage of sugar and probably appears as an intermediate in alcoholic fermentation" (Buchner & Meisenheimer, 1904, pp. 420–1). This led him to propose that alcoholic fermentation was a two-step process, with zymase catalyzing the reaction from glucose to lactic acid and another enzyme, lactacidase, catalyzing the reaction from lactic acid to alcohol.

The proposal that lactic acid figures in alcoholic fermentation was particularly interesting because it was the product of a different process, that associated with the souring of milk, which Pasteur (1858; 1857) had also investigated and interpreted as analogous to alcoholic fermentation. As well, Emil du Bois-Reymond (1859) had discovered the presence of lactic acid after muscle contraction or after death of an animal. The linkage between lactic fermentation and muscle action was a continuing focus of research in succeeding decades. The linkage was not well-established, though, until Fletcher and Hopkins (1907) showed that the formation of lactic acid during anaerobic muscle contraction was followed by its removal in the aerobic phase.

Lactic acid, however, was soon discredited as an intermediary in alcoholic fermentation on the grounds that adding it to yeast failed to generate alcohol (Slator, 1906). The nature of the relation between alcoholic fermentation and lactic fermentation was not revealed until later. Research seeking intermediaries of alcoholic fermentation turned rather to several three-carbon sugars which had been identified by organic chemists who had attacked glucose with alkalis – methylglyoxal, glyceraldehyde, and dihydroxyacetone. As well, Otto Neubauer, while investigating amino acid metabolism, identified pyruvic acid as an intermediary in that process and proposed it also figured in alcoholic fermentation (Neubauer & Fromherz, 1911). Other researchers quickly corroborated this finding and determined that it was decarboxylated to yield acetaldehyde.

The emerging challenge for biochemists was both to determine which of the possible intermediates figured in the fermentation pathway and to develop a model of the pathway that related those that did occur using only known chemical operations such as oxidations, reductions, and decarboxylations. Carl Neuberg developed a comprehensive model of a sequence of reactions for generating alcohol from glucose (Neuberg & Kerb, 1914). As shown in Figure 3.6, he proposed that glucose was scissioned into two molecules each of methylglyoxal and water. The methylglyoxal then reacted with acetaldehyde (produced in a previous iteration of the process) and water, generating both pyruvic acid and alcohol. The pyruvic acid was then decarboxylated to acetaldehyde. (Neuberg also proposed an alternative route by which methylglyoxal would generate glycerol and pyruvic acid, which could then be decarboxylated to provide the initial quantity of acetaldehyde.) Neuberg's model